Compositions of AMP-18 isolated from mouse and pig antrum tissue stimulate growth of confluent stomach, intestinal, and kidney epithelial cells in culture; human, monkey, dog and rat cells are also shown to respond. This mitogenic (growth stimulating) effect is inhibited by specific antisera (antibodies) to AMP-18, supporting the conclusion that AMP-18, or its products, *e.g.* peptides derived from the protein by isolation of segments of the protein or synthesis, is a growth factor. Indeed, certain synthetic peptides whose amino acid sequences represent a central region of the AMP-18 protein also have growth-factor activity. The peptides also speed wound repair in tissue culture assays, indicating a stimulatory effect on cell migration, the process which mediates restitution of stomach mucosal injury. Thus, the protein and its active peptides are motogens. Unexpectedly, peptides derived from sub-domains of the parent molecule can inhibit the mitogenic effect of bioactive synthetic peptides and of the intact, natural protein present in stomach extracts.

There are 3 activities of the gastrokine proteins and peptides of the present invention. The proteins are **motogens** because they stimulate cells to migrate. They are **mitogens** because they stimulate cell division. They function as **cytoprotective agents** because they maintain the integrity of the epithelium (as shown by the protection conferred on electrically resistant epithelial cell layers in tissue culture treated with damaging agents such as oxidants or non-steroidal anti-inflammatory drugs NSAIDs).

The invention relates a group of isolated homologous cellular growth stimulating proteins designated gastrokines, that are produced by gastric epithelial cells and include the amino acid sequence VKEK/QKKXXGKGPGGXPPPK (SEQ ID NO: 1). An isolated protein of the group has an amino acid sequence as shown in FIG. 7. The protein present in pig gastric epithelia in a processed form lacking the 20 amino acids which constitute a signal peptide sequence, has 165 amino acids and an estimated molecular weight of approximately 18kD as measured by polyacrylamide gel electophoresis. Signal peptides are cleaved after passage through endoplasmic reticulum (ER). The protein is capable of being secreted. The amino acid sequence shown in FIG. 3 was deduced from a human cDNA sequence. An embodiment of the protein is shown with an amino acid sequence as in FIG. 6, a sequence predicted from mouse RNA and DNA.

A growth stimulating (bioactive) peptide may be derived from a protein of the gastrokine group. Bioactive peptides rather than proteins are preferred for use because they are smaller, consequently the cost of synthesizing them is lower than for an entire protein.

In addition, a modified peptide may be produced by the following method:

(a) eliminating major protease sites in an unmodified peptide amino acid sequence by amino acid substitution or deletion; and/or

(b) introducing into the modified amino acid analogs of amino acids in the unmodified peptide.

An aspect of the invention is a synthetic growth stimulating peptide, having a sequence of amino acids from positions 78 to 119 as shown in FIG. 3.

Another peptide has a sequence of amino acids from position 97 to position 117 as shown in FIG. 3.

Another peptide has a sequence of amino acids from position 97 to position 121 as shown in FIG. 3.

Another peptide has a sequence of amino acids from position 104 to position 117 as shown in FIG. 3.

An embodiment of an isolated bioactive peptide has one of the following sequences: LDTMVKEQK..GKGPGGAPPKDLMY (SEQ ID NO: 2) or KKLQGKGPGGPPPK (SEQ ID NO: 3) . An embodiment of an inhibitor of a protein of the gastrokine group has the amino acid sequence KKTCIVHKMKK (SEQ ID NO: 4) or KKEVMPSIQSLDALVKEKK (SEQ ID NO: 5) . (see also Table 1)

The invention also relates a pharmaceutical composition including at least a growth stimulating peptide.

A pharmaceutical composition for the treatment of diseases associated with overgrowth of gastric epithelia, includes an inhibitor of a protein of the group of gastrokines or of a growth stimulating peptide derived from the gastrokine proteins.

A pharmaceutical composition for the treatment of diseases of the colon and small intestine includes at least a growth stimulating peptide of the present invention. Examples of such diseases include ulcerative colitis and Crohn's Disease.

(b) providing environmental conditions allowing migration of the epithelial cells.

A method for cytoprotection of damaged epithelial cells in the gastrointestinal tract of mammals, includes the following steps:

(a) contacting the damaged epithelial cells with a composition including a protein of the gastrokine group or a peptide derived from the protein; and

(b) providing environmental conditions allowing repair of the epithelial cells.

The damaged cells may form an ulcer.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a human genomic nucleotide sequence (SEQ ID NO: 11) of a pre-gastrokine; sequence features were determined from cDNA and PCR of human genomic DNA amph-ge8.seq Length: 7995 predicted promoter: 1405; exon 1: 1436-1490; exon 2: 4292-4345; exon 3: 4434-4571; exon 4: 5668-5778; exon 5: 6709-6856; exon 6: 7525-7770; polyA site: 7751.

FIG. 2 is a human cDNA sequence (SEQ ID NO: 12); the DNA clone was obtained by differential expression cloning from human gastric cDNA libraries.

FIG. 3 is a human preAMP-18 protein sequence (SEQ ID NO: 13) predicted from a cDNA clone based on Powell (1987) and revised by the present inventors; N-21 is the expected N-terminus of the mature protein.

FIG. 4 is a mouse preAMP-18 sequence (SEQ ID NO: 14) determined from RT-PCR of mRNA and PCR of BAC-clones of mouse genomic DNA sequences:

predicted promoter: 1874 experimental transcription start site: 1906 translation initiation site: 1945 CDS 1: 1906-1956; CDS 2: 3532-3582; CDS 3: 3673-3813; CDS 4: 4595-4705; CDS 5: 5608-5749; CDS 6: 6445-6542; polyA site: 6636.

FIG. 5 is a mouse cDNA sequence (SEQ ID NO: 15) for preAMP-18.

FIG. 6 is mouse preAMP-18 amino acid sequence (SEQ ID NO: 16); RT-PCR performed on RNA isolated from mouse stomach antrum: Y-21 is the predicted N-terminus of the mature protein; the spaces indicated by .. mean there are no nucleotides there to align with other sequences in FIG. 11.

FIG. 7 is a [pig genomic DNA related to the cDNA] cDNA (SEQ ID NO: 17) expressing porcine AMP-18.

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[FIG. 8 is the cDNA pig sequence of AMP-18. *Based on Powell (1987). **D**-21 is the N-terminus of the mature protein - confirmed by sequencing of the protein isolated from pig stomach.]

FIG. [9] 8 is pig pre-gastrokine (pre-AMP-18) protein sequence (SEQ ID NO: 18) predicted from cDNA clone based on Powell (1987) **D**-21 is the N-terminus of the mature protein - confirmed by sequencing of the protein isolated from pig stomach.

FIG. [10] 9 is a comparison between the amino acid sequences of human (SEQ ID NO: 13) versus pig (SEQ ID NO: 18) pre-gastrokine.

FIG. [11] 10 shows a computer-generated alignment comparison of human (SEQ ID NO: 13), pig (SEQ ID NO: 18) and mouse (SEQ ID NO: 16) predicted protein sequences determined from sequencing of cDNA clones for human and pig AMP-18, and by polymerase chain reaction of mouse RNA and DNA using preAMP-18 specific oligonucleotide primers; in each case the first 20 amino acids constitute the signal peptide, cleaved after passage through the endoplasmic reticulum membrane.

FIG. [12] 11 shows the effect of porcine gastric antrum mucosal extract, human AMP peptide 77-97, and EGF on growth of gastric epithelial cells; AGS cells were grown in DMEM containing fetal bovine serum (5%) in 60-mm dishes; different amounts of pig antrum extract, HPLC purified peptide 77-97, and/or EGF were added; four days later the cells were dispersed and counted with a hemocytometer; antrum extract and peptides each stimulated cell growth in a concentration-dependent manner; the bar graph shows that at saturating doses, peptide 77-97 (8g/ml) or EGF (50ng/ml) was mitogenic; together they were additive suggesting that the two mitogens act using different receptors and/or signaling pathways; anti-AMP antibodies inhibited the antrum extract but did not inhibit peptide 77-97.

FIG. [13] 12 shows the structure of the human and mouse preAMP-18 genes; the number of base pairs in introns are shown above the bars; exons are indicated E1-E6 and introns I1-I5; there are minor differences in intron length.

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TABLE 1: BIOACTIVITY OF SYNTHETIC PEPTIDES BASED ON THE SEQUENCE OF GASTROKINE (AMP-18)

Name of Peptide, Sequence in Human	#AA	AMINO ACID SEQUENCE	$K_{1/2}$,μM
78-119	42	KKTCIVHKMKKEVMPSIQSLDALVKEKKLQGKGPGGPPPKGL (SEQ ID NO: 6)	0.3
78-88	11	KKTCIVHKMKK (SEQ ID NO: 4)	Inactive
87-105	19	KKEVMPSIQSLDALVKEKK (SEQ ID NO: 5)	Inactive
104-117	14	**KKLQGKGPGGPPPK**(SEQ ID NO: 3)	0.8
104-11[illegible]	18	**KKLQGKGPGGPPPKGLMY** (SEQ ID NO: 7)	1.0
97-117	21	LDALVKEKKLQGKGPGGPPPK(SEQ ID NO:8)	0.3
97-117**	21	GKPLGQPGKVPKLDGKEPLAK (SEQ ID NO:9)	Inactive
97-121	25	LDALVKEKKLQGKGPGGPPPKGLMY (SEQ ID NO:10)	0.2
109-117	9	KGPGGPPPK(portion of SEQ ID NO:10)	2.5
104-109	6	KKLQGK (portion of SEQ ID NO:10)	7.4
110-113	4	GPGG (portion of SEQ ID NO: 10)	Inactive
mouse 97-119	23	LDTMVKEQK..GKGPGGAPPKDLMY (SEQ ID NO:2)	0.2

Table 1: Analysis of mitogenic peptides derived from the human and mouse gastrokine (AMP-18) sequence. A 14 amino acid mitogenic domain is in bold type. *Peptides are identified by their position in the amino acid sequence of the pre-gastrokine (preAMP-18). #AA; number of amino acids in a peptide. $K_{1/2}$; concentration for half-maximal growth stimulation.

Overlapping inactive peptides can inhibit the activity of the mitogenic peptides: that is, human peptides 78-88 and 87-105 block the activity of peptide 78-119, and while peptide 87-105 blocks the activity of peptide 104-117, the peptide 78-88 does not. Peptides 78-88 and 87-105 block the activity of the protein in stomach extracts.

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